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Fungal Communities across an Edaphic Gradient in Central Borneo †

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Abstract: To examine the role of soil properties in influencing tropical fungal communities, soils were collected from Barito Ulu in central Borneo across an edaphic gradient ranging from clay-rich ultisols to sandy spodosols and from the upper and lower horizons and were subjected to high-throughput Illumina sequencing of the ITS1 region. The fungal community was clearly distinct between contrasting soils and depths, but the diversity metrics did not show significant differences. Differentiation by depth was more marked as the soils became less fertile. There were few marked impacts on fungal phyla or functional guilds at a broad level, but Ascomycota were more abundant in less acidic soils with a narrower C:N ratio. The forests of South-east Asia remain an underexplored frontier for fungal diversity studies.

Keywords: acrisol; carbon; ectomycorrhizas; eDNA; fungi; *kerangas*; podzol

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1. Introduction

Whilst our understanding of the patterns and drivers of fungal community composition is increasing, we have a paucity of data from tropical regions that needs addressing due to the key role fungi play in ecosystem processes [1] and their importance in contributing to high overall tropical biodiversity [2]. Molecular surveys of tropical soil fungi are increasing, but much of the data are from Amazonia [3–7], with South-east Asia being less explored [8,9]. Soil chemical properties are known to play an important role in structuring fungal communities, with pH and soil carbon being noted as particularly important [7], along with soil phosphorus [5] and calcium [10]. Furthermore, we know that soil depth can influence the fungal community, at least partly, due to the separation of the soil depth niche by saprotrophic and ectomycorrhizal fungi, with saprotrophs being stronger competitors in recently shed leaf litter [3,11]. The island of Borneo is a valuable study system in this regard, as it contains high pedodiversity at local scales, which is known to influence tree communities [12,13]. However, how this pedodiversity influences soil microbes, and especially fungi, is poorly explored [14]. In this study, we examined the fungal community composition in soil across edaphic gradients using Illumina sequencing, presenting a detailed study of soil fungi in the tropical forests of Borneo.

2. Methods

Soils were collected from the Project Barito Ulu research area in central Borneo (114°0′ E, 0°6′ S), which has a mean annual precipitation of 3800 mm [15] and is located at an elevation range of about 150 to 200 m a.s.l. Whilst the geology is based on a Tertiary sedimentary formation, the research area contains a range of forest types developed on contrasting soils, from more clay-rich udult ultisols (hosting a tall lowland evergreen rain forest (LERF2; *sensu* Proctor [16])) and grading through sandy humult ultisols (hosting a shorter lowland evergreen rain forest: LERF1) to spodosols hosting heath forests (HF1 and

HF2) (*kerangas*). From each forest type, soil samples were collected 0–5 cm from the surface and deeper, at a depth of 15–20 cm, after removing the superficial litter layer.

The number of fungal colony-forming units (CFUs) was determined by adding soil to sterile water in a 1:10 ratio and then serially diluting it and adding it to potato dextrose agar (with 0.05% (*w*/*v*) chloramphenicol) in Petri dishes before incubation for 96 h at 25 °C [17].

DNA was extracted from soil using a MoBio PowerSoil DNA Extraction Kit with one minute in a FastPrep 120 during the first stage. To amplify the fungal ITS1 region, the primers ITS5 and 5.8S_fungi [18] were used for PCR and sequencing on an Illumina HiSeq 2500 with 2 × 300 bp paired-end sequencing following the protocol in Brearley [19]. Basecalling and de-multiplexing were performed using CASAVA v.1.8.2. The primers and adapter sequences were trimmed using Cutadapt v.1.2.1 [20], and low-quality bases were removed using Sickle v. 1.200 [21]. FLASH v.1.2.8 [22] was used to assemble each pair of reads into a single sequence. Sequences shorter than 200 bp or longer than 600 bp were removed using a custom script, as were those matching PhiX. Clustering of sequences (at 99%), removal of chimeras, and defining OTU abundances were carried out in USEARCH7 [23]. After OTU-picking, the taxonomic assignment of each OTU was carried out using the QIIME [24] script *assign_taxonomy.py* and using the RDP classifier [25] to match a representative sequence from each OTU to a sequence from the UNITE ITS database [26] at 97% similarity. Sequences not matching the Kingdom Fungi were removed from further analysis.

Soil pH was measured in a 1:2.5 soil:water ratio after 1 hr of equilibration using a Sartorius PB-11 pH meter, and total carbon and nitrogen were analysed using a Vario ELCube elemental analyser.

Functional guilds based on trophic mode were assigned using FUNGuild [27]: only guild assignments where the confidence ranking was "highly probable", "probable" or "possible" were used. Krona [28] was used to visualize fungal taxa, an NMDS ordination was conducted using Vegan [29] in R, and general linear models (GLMs) and correlations were conducted in Minitab v.19.

3. Results

Fungal CFUs were significantly more abundant in the surface (0–5 cm) soil layers than in deeper (15–20 cm) layers (GLM: $F = 9.1$, $p = 0.013$), with this difference being more marked for the spodosol and humult soils than the udult soil (GLM: $F = 6.6$, $p = 0.015$) (Figure 1).

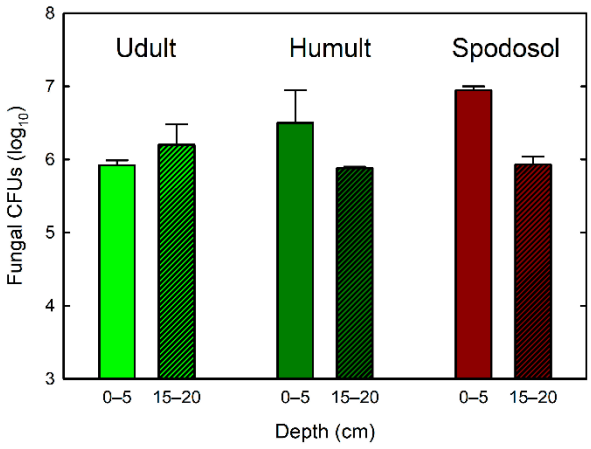

Figure 1. Abundance of fungal colony-forming units (CFUs) (mean ± standard error) across different soil types and depths at Barito Ulu, central Borneo.

A total of 8536 fungal OTUs were found, of which 4197 could not be assigned to a phylum. Of those that could be assigned to a phylum, Ascomycota dominated the community with 76% of the assigned sequences followed by Basidiomycota (23%) and

Glomeromycota (<1%). Other phyla, such as Chytridiomycota, Neocallimastigomycota, and Zygomycota, totaled less than 1% of all of the sequences (Figure 2). The most abundant Ascomycota classes assigned were Sordariomycetes (38%), Eurotiomycetes (21%), and Archaeorhizomycetes (4.7%). In Basidiomycota, the most abundant orders were Agaricales, Russulales, and Catharellales (which contain a large number of ectomycorrhizal families between them) (Figure 2). The majority of sequences (70%) could not be confidently assigned to a functional guild, but of those that could, Symbiotroph was the most abundant guild (14%) followed by Pathotroph–Saprotroph–Symbiotroph (6.4%) and then Saprotoph (3.5%). There was no significant influence of either soil type or depth (or their interaction) on the relative abundance of any of the phyla or functional guilds (GLM: $p > 0.10$ in all cases).

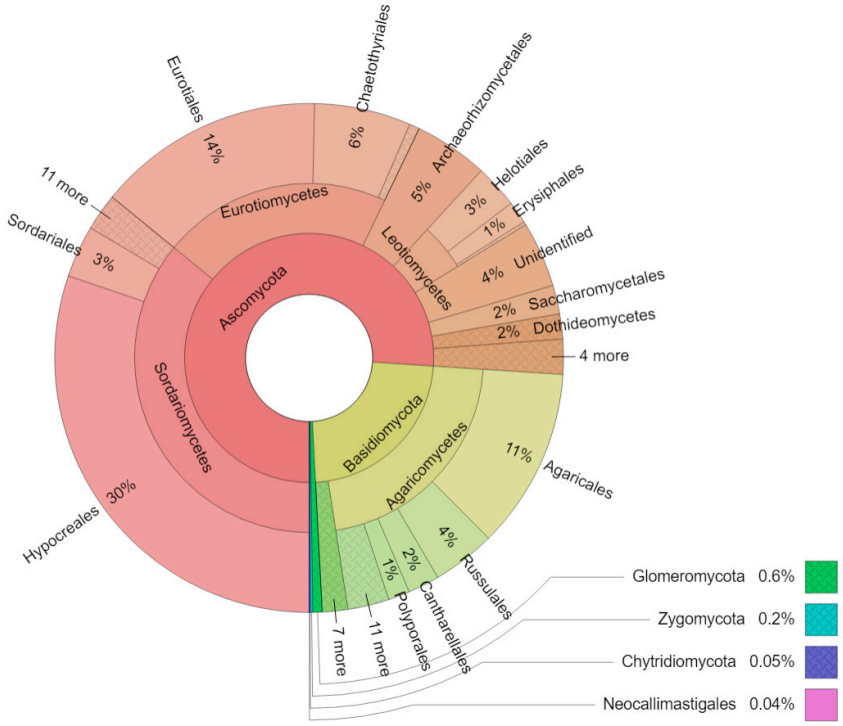

Figure 2. Abundance of fungal OTU sequences by dominant phyla, orders, and classes across all samples from different soil types and depths at Barito Ulu, central Borneo.

There was a mean of 784 (±s.d. 269) OTUs per sample and a mean Chao1 estimator of 1536 (±s.d. 451) OTUs per sample. There was no indication of either of these diversity metrics differing by soil type or depth (GLM: $p > 0.10$). However, there was a clear separation of the fungal communities in the NMDS ordination plot (Figure 3), with the *x*-axis representing a soil type gradient, and the *y*-axis representing soil depth differentiation that was more marked in the spodosol than in the ultisols.

Surface soils were significantly richer in carbon (C) and nitrogen (N) but were more acidic than the deeper soils, with increasing C and N and decreasing acidity throughout the udult ultisol to spodosol sequence (Table 1); the differences between the surface and deeper soils were more marked for the humult and spodosol soils then for the udult soil (Table 1). There were few correlations between soil properties and fungal richness/diversity or in the proportional abundance of guilds and phyla, with the exception of there being a declining abundance of Ascomycota in more acidic soils ($\rho = 0.47$, $p = 0.072$) with greater C ($\rho = -0.46$, $p = 0.07$) and wider C:N ratios ($\rho = -0.53$, $p = 0.035$) and a greater number of fungal CFUs in acidic C- and N-rich soils ($|\rho| = 0.55$–0.63, $p < 0.028$).

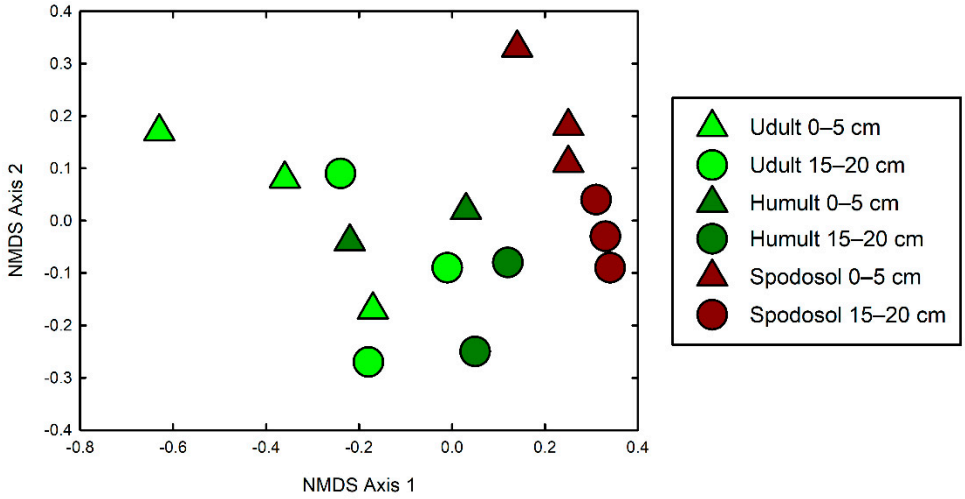

Figure 3. Ordination (NMDS) of fungal OTU sequences from different soil types and depths at Barito Ulu, central Borneo.

Table 1. Soil parameters (mean ± standard error) from different soil types and depths at Barito Ulu, central Borneo. Under each parameter, significance codes from a GLM are presented for soil type (S), depth (D), and their interaction (S × D) as *** $p < 0.001$, **, $p < 0.01$, or $^{n.s.}$ = not significant.

Parameter	Soil Type	Depth (cm)	
		0–5	15–20
pH S ***, D ***, S × D **	Udult	3.36 ± 0.08	3.55 ± 0.09
	Humult	2.81 ± 0.04	3.63 ± 0.05
	Spodosol	2.76 ± 0.07	3.14 ± 0.07
N (%) S ***, D ***, S × D $^{n.s.}$	Udult	0.27 ± 0.10	0.09 ± 0.02
	Humult	1.11 ± 0.08	0.15 ± 0.02
	Spodosol	1.25 ± 0.09	0.17 ± 0.04
C (%) S ***, D ***, S × D **	Udult	4.09 ± 0.69	1.07 ± 0.10
	Humult	39.8 ± 4.95	2.31 ± 0.29
	Spodosol	48.9 ± 1.05	4.71 ± 1.36

4. Discussion

I show here that soils can influence the fungal communities inhabiting them through differences in their chemical and textural properties, and some aspects of the fungal community correlate with soil chemical parameters at the individual sample level. These patterns are all found over a small spatial scale (less than 1 km^2) within a mosaic of soil and forest typologies (F.Q. Brearley and J. Proctor, unpublished), contrasting with other similar studies that have examined forests and soils over a broader geographical extent [4,7,14]. In common with other studies [7,14], the forests over spodosols (also known as *kerangas* or white sand forests) showed a distinctive fungal community composition from those in more clay-rich udult ultisols, but, in this study, humult ultisols were the intermediate between these two, showing a continuum of responses to the edaphic environment.

Whilst there were clear influences of both soil type and soil depth on the fungal community overall, it was difficult to elucidate a clear influence on individual fungal phyla or functional guilds. Additionally, soil type did not influence fungal alpha diversity, corroborating the results of Tripathi et al. [14] in other Bornean heath forests although contrasting with Vasco-Palacios et al. [7], who found lower fungal diversity in Amazonian white sand soils. However, Tripathi et al. [14] did find greater beta diversity in ultisols, which they suggested might be due to the more diverse plant communities in the lowland evergreen rain forests on these soils compared to the white sand forests on spodosols. This

is supported by the results of Peay et al. [4], who found plant and fungal beta diversity to be correlated across the forests of western Amazonia.

At the phylum level, Tripathi et al. [14] found Ascomycota to be the least common in dipterocarp forests (analogous to our udult ultisol), which is in contrast with this study, where they were the most common in this soil type (albeit not significantly) and became less abundant in more acidic soils with wider C:N ratios. Although dominant, Ascomycota have a range of stress-tolerance genes [30], and the greater recalcitrance of soil carbon in the spodosols may be a factor leading to their reduced abundance due to competition with Basidiomycota, which have a greater capacity to degrade more complex organic substrates [31,32]. The relatively high abundance of Archaeorhizomycetes found here agrees with other work from Amazonia [7]. The large proportion of OTUs that could not even be assigned to a phylum was surprising and could represent either bioinformatic issues or a number of unknown fungal lineages remaining to be discovered [19] in this underexplored biodiversity hotspot.

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